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Practitioner's Docket No. **100788.0017PRO**

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Fareed, Kureshy and Mahant, Vijay

For: Analytic System

Box Provisional Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

COVER SHEET FOR FILING PROVISIONAL APPLICATION (37 C.F.R. § 1.51(c)(1))

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.51(c)(1)(i). The following comprises the information required by 37 C.F.R. § 1.51(c)(1):

1. The following comprises the information required by 37 C.F.R.§ 1.51(c)(1):

2. The names of the inventors are (37 C.F.R. § 1.51(c)(1)(ii)):

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   2. Vijay Mahant

3. Residence addresses of the inventors, as numbered above (37 C.F.R. § 1.51(c)(1)(iii)):

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Date: 5/28/02

Erika Simpson

4. The title of the invention is (37 C.F.R. § 1.51(c)(1)(iv)):

Analytic System

5. The name, registration, customer and telephone numbers of the practitioner are (37 C.F.R. § 1.51(c)(1)(v)):

Name of practitioner:	Robert D. Fish
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6. The docket number used to identify this application is (37 C.F.R. § 1.51(c)(1)(vi)):

Docket No. 100788.0017PRO

7. The correspondence address for this application is (37 C.F.R. § 1.51(c)(1)(vii)):

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8. Statement as to whether invention was made by an agency of the U.S. Government or under contract with an agency of the U.S. Government. (37 C.F.R. § 1.51(c)(1)(viii)).

This invention was NOT made by an agency of the United States Government, or under contract with an agency of the United States Government.

9. Identification of documents accompanying this cover sheet:

A. Documents required by 37 C.F.R. § 1.51(c)(2)-(3):

Specification:	No. of pages	12
Drawings:	No. of sheets	1

B. Additional documents:

None

10. Fee

The filing fee for this provisional application, as set in 37 C.F.R. § 1.16(k), is $80.00 for a small entity.

Applicant is a small entity.

11. Small entity assertion

Small entity status is asserted for this application by payment of the small entity filing fee under § 1.16(k). 37 C.F.R. § 1.27(c)(3).

12. Fee payment

Fee payment in the amount of $80.00 is being made at this time.

13. Method of fee payment

Check in the amount of $80.00.

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ANALYTIC SYSTEM

Field of The Invention

The field of the invention is micro array systems, and particularly automated micro array systems.

Background of The Invention

Recent advances in genomics and proteomics research made numerous nucleotide and peptide sequences available, necessitating high-throughput screening of samples for presence and/or quantity of genes and/or gene expression. While automation of individual steps (*e.g.*, DNA isolation, protein fractionation, etc.) in high-throughput screening may be performed using relatively simple instrument configurations, integration of multiple and distinct steps in automated high-throughput screening remains problematic.

For example, sample analysis for detection and quantification of one or more analytes may be performed in nano-volumes on a single chip (see *e.g.*, "Lab-on-a-chip" from Agilent or Caliper Technologies). Such multiple analyte detection can advantageously be performed in relatively short time using minimal amounts of sample. Moreover, all steps from handling of the sample after application of the sample to detection and analysis are performed within the same device. However, identification and quantification of the detected analyte using nanoelectrophoresis is typically restricted to the size of the analyte. Moreover, resolution of individual analytes becomes increasingly difficult as the size or charge difference between the analytes decreases. Consequently, such nanoelectrophoretic systems are generally limited to characterization of an analyte by its molecular weight.

Where high resolution of molecular weights of an analyte is particularly important, analysis of complex samples may be coupled with laser desorption - time of flight mass spectroscopic analysis (see *e.g.*, Ciphergen Biosystems' LD-TOF multi-analyte desorption chips, or Sequenome chip). Here, components of a complex sample are immobilized on a carrier chip (*e.g.*, chip with anion exchange resin or hydrophobic interaction resin) and subjected to size analysis after desorption according to their molecular properties in an analysis system. LD-TOF coupled analysis is typically highly sensitive and often requires

only minimal sample preparation. Moreover, LD-TOF coupled analysis provides relatively high resolution among particular analytes. However, identification of particular analytes is still mostly limited to size determination.

Alternatively, and especially where the analyte is a DNA or RNA, various formats of automated modular PCR-based analysis are known in the art. For example, where a single sample is analyzed for presence or absence of a particular sequence, all or almost all of the reagents and sample may be introduced into an automated system from a single cartridge (see *e.g.*, Cepheid's i-CORE system). On the other hand, and especially where a relatively high number of samples are concurrently analyzed, a full robotic PCR station may be employed (see *e.g.*, Orchid Biocomputer SNP Analysis system). Such systems typically provide an analysis procedure that integrates sample manipulation with nucleic acid amplification and product analysis. However, automated modular PCR-based analysis typically rely on amplification of target DNA to generate appreciable signals, thereby introducing significant complexity and numerous error-prone procedures. Moreover, while PCR based systems are frequently operated in a dedicated environment using dedicated equipment to prevent non-sample specific signals, problems associated with contamination via sample carry-over may still persist. Thus, automated modular PCR-based analysis tends to be highly expensive, and is generally limited to exclusive analysis of nucleic acids.

In still further examples, nucleic acid-containing samples can be analyzed by their hybridization characteristics with at least partially complementary and immobilized nucleic acids, thereby providing quantitative and qualitative information on a particular sample. Hybridization of a nucleic acid to corresponding solid-phase immobilized nucleic acids may be controlled by variation of temperature and/or ionic strength of the environment of the nucleic acid hybrid, and there are numerous systems known in the art.

For example, high density arrays of immobilized oligonucleotides on a silicon chip may be exposed to a sample containing nucleic acids that are complementary to at least some of the immobilized oligonucleotides (see *e.g.*, Affymetrix' GeneChip system). In such systems, a processed sample (typically a labeled and biotinylated in-vitro transcript of a previously prepared cDNA) is provided to the chip in a fluidics station that further controls

flow of reagents and hybridization temperature. After complementary labeled nucleic acids have hybridized to the corresponding nucleic acids on the chip, the chip is removed from the fluidics station and manually transferred to a scanner station in which the sample is analyzed via detection of the fluorescent labels. While such analytic devices typically allow a user to determine identity, presence, and/or quantity of a vast number of DNA/RNA analytes in a sample, substantial sample preparation (typically several hours to more than one day) and hybridization times (*e.g.*, about 16 hours at 40°C) are frequently necessary. Moreover, analytes detected and quantified using such systems tend to be limited to nucleic acids.

Alternatively, sample capture and hybridization may be controlled via electrostatic forces (see *e.g.*, Nanogen's NanoChip system). In such systems, capture probes and hybridization conditions may be individually controlled, thereby allowing custom addressing of individual analyte pixels. However, due to the complexity of loading and reading procedures, the analytic process is split among at least two independent devices: Analytes are typically bound in a loader section, while a reader (*i.e.*, array processor and scanner) will perform the readout of the sample.

In another system, detection may be performed using an electronic chip that provides a signal upon binding of a signaling oligonucleotide to an analyte oligonucleotide that is bound to a corresponding oligonucleotide that is immobilized on the chip (see *e.g.*, Motorola's iSensor system). While electronic detection and quantification may provide at least some advantages, most of such systems are prone to non-specific false-positive and/or false-negative signals due to contamination. Moreover, analytes detected and quantified using such systems tend to be limited to nucleic acids.

Thus, although various systems for micro array systems are known in the art, numerous problems still remain. Among other things, while various systems may provide at least some automation, fluid handling and sample detection/quantification of analyte binding are typically operated in separate devices, thus requiring at least some user intervention after the sample is applied to the system. Furthermore, all or almost all of the known micro array systems are limited to analysis of either nucleic acids or peptides. Therefore, there is still a need for an improved methods and systems for automated analytic devices.

Detailed Description

The inventors contemplate an analytic system (Infiniti™ system), which provides an operator with the capability to quantitatively and qualitatively analyze one or more analytes in a sample with minimum manual intervention. While known systems typically separate sample and/or analyte handling (*e.g.*, application, washing, hybridization) from analyte detection, the contemplated system will perform sample and/or analyte handling and analyte detection in an automated and preferably continuous manner.

Contemplated systems will typically include the following subsections: Sample and reagent handling, disposable handling, microarray processing, stringency station, optical detection system, waste handling, and data/result analysis. A perspective schematic of one exemplary analytic system is depicted in **Figure 1**.

These sections handle all the processing required for DNA and Proteomic analysis for the following not exclusive list: SNP and STR analysis, microsatellite analysis, gene- and protein expression analysis, and protein quantification and identification.

In particularly preferred aspects, one or more of the following processing steps may be included in contemplated analytic systems: Sample and reagent dispensing operations, analysis of environmental conditions for sample and reagent, wash processes using aspiration dispenser, ultrasonic energy and heat, use of ultrasonic energy for mixing to improve hybridization and binding, bar code for reagent tracking and sample identification, sonic irradiation for chip surface detection, laser irradiation for volume and surface detection, and the use of a reagent module as a communication link between at least two of manufacturing, assay development scientist, user and technical support without any intervention from the operator.

In further particularly contemplated aspects, stringency is preferably controlled at least in part determined by heat (*e.g.*, thermal stringency for improving specificity), and optical detection employs a two wavelength system for excitation and detection using confocal microscope technology. Assay development software is contemplated to assist in automation of new tests.

In yet further contemplated aspects, the software/operator interface will provide the analytic system with specific test requirement for a specific sample, which may be downloaded from a host computer. Furthermore, it is contemplated that the interface may also be used to transmit results through web or modem. Thus, a contemplated operation communication link may include an integrated color monitor, a mouse and keyboard, a R/W CD, 40 GB of hard drive, and bioinformatics software for data/result analysis.

Therefore, in one aspect of the inventive subject matter, an analytical device will include a multi-reagent pack with a housing that includes a container and a memory element and a data transfer interface that receives data from the memory element and provides at least some of the data to a person other than an operator of the analytical device. The housing may further include a second container, and a closing element may be coupled to the housing and movable between a first and a second position, wherein the multi-reagent pack is configured such that the closing element is moved between the first and second position by an actuator when the multi-reagent pack is disposed within the analytical device.

Preferred memory elements comprise a read-only memory, and it is contemplated that the data transfer interface transfers the at least some of the data via an Internet, wherein the person other than the operator is in a remote location relative to the operator of the analytical device. Alternatively, the memory element may comprise a random access memory, wherein the data are associated with a history of the multi-reagent pack. The history may include a parameter selected from the group consisting of composition of a reagent in the container, volume of a reagent in the container, environmental data collected within the analytical device, number of access to a reagent in the container, and type of test performed using the analytical device, and it is further contemplated that the person other than the operator uses the data to initiate delivery of a replacement multi-reagent pack. In still further preferred aspects, the person other than the operator uses the data to identify an event associated with a test performed using the analytical device.

Thus, a multi-reagent pack may comprise a housing that includes a plurality of containers, wherein a first container of the plurality of containers contains a first reagent and wherein a second container of the plurality of containers contains a second reagent; a closing

element coupled to the housing and movable between a first and a second position; wherein the plurality of containers are closed when the closing element is in the first position, and wherein at least the first and second containers are accessible to a fluid receiving mechanism when the closing element is in the second position; and wherein the multi-reagent pack is configured such that the closing element is moved between the first and second position by an actuator when the multi-reagent pack is disposed within an analytic device.

Preferred first reagent is employed in a first test wherein the second reagent is employed in a second test, and wherein the first and second test are performed in the analytic device, wherein the first test comprises at least one of detection and quantification of a nucleic acid, and wherein the second test comprises at least one of detection and quantification of a peptide.

The closing element is preferably slidably coupled to the housing, and the fluid receiving mechanism comprises an automatic pipette. The multi-reagent pack may further comprise a memory element that stores a plurality of data, wherein the memory element comprises a random access memory, and wherein the data are associated with a history of the multi-reagent pack. Preferred histories include a parameter selected from the group consisting of composition of a reagent in the container, volume of a reagent in the container, environmental data collected within an analytical device, number of access to a reagent in the container, and type of test performed using the analytical device. It is further especially preferred that the memory element is configured to cooperate with a data transfer interface that receives the data from the memory element and provides at least some of the data to a person other than an operator of an analytical device that uses the multi-reagent pack.

Alternatively, a multi-reagent pack may include a housing that includes a plurality of containers, wherein a first container of the plurality of containers contains a first reagent and wherein a second container of the plurality of containers contains a second reagent; a movable closing element coupled to the housing, wherein the housing is configured such that the multi-reagent pack is disposed within an analytical device during use; and a memory element coupled to the housing that contains data associated with a parameter selected from the group consisting of an activation code for the analytical device, environmental data

collected within the analytical device, supply information of the first reagent, composition information of the first reagent, and a deactivation code for the analytical device.

In such multi-reagent packs, the closing element is slidably coupled to the housing, and the memory element comprises a random access memory that cooperates with a data transfer interface that provides at least some of the data to a person other than an operator of the analytical device. Typically, the data transfer interface transfers the at least some of the data via an Internet, wherein the person other than the operator uses the data to initiate delivery of a replacement multi-reagent pack, and it is further preferred that the person other than the operator uses the data to identify an event associated with a test performed using the analytical device.

In a further preferred aspect of the inventive subject matter, an analytical device may include a biochip at least partially disposed on a platform, and a plurality of reagents in a plurality of containers; a liquid manipulator movable along an x-axis, a y-axis, and a z-axis and coupled to a first actuator that moves the liquid manipulator along the y-axis and z-axis; a non-liquid manipulator movable along an x-axis, a y-axis, and a z-axis and coupled to a second actuator that moves the liquid manipulator along the y-axis and z-axis; wherein the liquid manipulator transfers at least one of the reagents from at least one of the containers to the biochip, wherein the non-liquid manipulator moves the biochip from a first position to a second position; and wherein the liquid manipulator and the non-liquid manipulator are moved along the x-axis using the first actuator. Contemplated biochips will preferably include a plurality of samples selected from the group consisting of a nucleic acid sample, a peptide sample, a pharmacologically active compound, and an epitope.

It is further preferred that the liquid manipulator comprises an automatic pipette, wherein the automatic pipette uses a disposable pipette tip that is removed at least in part by the non-liquid manipulator. Further contemplated first actuator includes a step motor, and the second actuator includes a step motor, wherein the second actuator moves the non-liquid manipulator in a circular motion.

The non-liquid manipulator preferably retrieves the biochip from a magazine disposed within the analytical device, wherein the non-liquid manipulator moves the biochip

from the platform to an analysis section comprising at least one of a confocal microscope and a photomultiplier.

Suitable analytical devices may also comprise a data transfer interface, wherein the data transfer interface couples the analytical device with a person other than the operator of the analytical device (e.g., via an Internet). Therefore, it should be recognized that the person other than the operator may be in a remote location relative to the analytical device, and in still further preferred embodiments, the data transfer device receives at least some of the data from a memory element that is coupled to at least one of the containers.

In still further contemplated aspects, an analytical device may include a sample processing platform that receives a biochip that is at least partially immersed in a fluid, wherein the biochip binds an analyte from the fluid and wherein the fluid further comprises a non-analyte; an energy source functionally coupled to the platform or the biochip, wherein the energy source delivers energy to the fluid in an amount effective to (a) allow binding of the analyte to the biochip and (b) prevent binding of the non-analyte; and wherein the biochip is transported within the analytical device to an analysis section while the analyte is bound to the biochip and the biochip is at least partially immersed in a second fluid. Contemplated aspects of especially suitable biochips are disclosed in copending PCT/US02/03917 and PCT/US01/47991, both of which are incorporated by reference herein. In further preferred aspects, the matrix layer(s) of the biochip may also comprise a hydrophobic polymer, or a hydrophilic polymer may be combined with a hydrophobic polymer. For example, columns of hydrophobic polymers may be combined with columns of hydrophilic polymers, wherein these columns may be adjacent to each other or within each other (*e.g.*, hydrophilic column fit inside a larger hydrophobic column).

It is especially contemplated that the sample processing platform is configured to receive a second biochip, and that the sample processing platform is coupled to the energy source and wherein the energy source comprises at least one of a heater and a cooling element. The sample processing platform may further be functionally coupled to the energy source and wherein the energy source comprises an ultrasound source, or the sample

processing platform may be functionally coupled to the energy source and wherein the energy source comprises an ultrasound source.

The biochip may comprise a metal base that cooperates with the sample processing platform to transmit the energy from the sample processing platform to the biochip, wherein the biochip may be in a substantially horizontal position when the biochip is disposed in at least one of the sample processing platform and the analysis section. It is further contemplated that the biochip is further transported from a magazine to the sample processing platform within the analytical device.

The sample may be applied to the biochip from a sample reservoir within the analytical device, and suitable devices may further include a data transfer interface, wherein the data transfer interface couples the analytical device with a person other than the operator of the analytical device (e.g., via an Internet). Consequently, the person other than the operator is in a remote location relative to the analytical device.

In additionally contemplated devices, the biochip is immersed in the fluid and wherein the analyte comprises a nucleic acid or a peptide. Therefore, the analyte may comprise a nucleic acid with a first sequence, wherein the non-analyte comprises a nucleic acid with a second sequence, and wherein the first and second sequence differ in at least one nucleoside.

Binding of the analyte may be selected from the group consisting of hybridization between a first and a second nucleic acid, non-covalent binding between a first and a second peptide, and non-covalent binding between a peptide and a non-peptide.

Further contemplated aspects of suitable devices include a pipette that comprises a fluid receiving element coupled to a vacuum source; an energy source and an energy detector operationally coupled to fluid receiving element; wherein the energy source provides an energy to a volume of a fluid disposed within the fluid receiving element, wherein the fluid reflects at least part of the energy provided by the energy source, and wherein the energy detector detects the reflected energy; and a processor operationally coupled to the energy

detector that calculates the volume of the fluid in the fluid receiving element using the detected reflected energy.

The fluid receiving element (e.g., disposable pipette tip) may be detachable from the vacuum source, and may have a working volume of equal or less than 200 microliter. Contemplated vacuum sources will include a vacuum pump coupled to a stepping motor, and suitable energy sources may comprise a laser or an ultrasound transmitter, and wherein the energy detector comprises a photodiode or an ultrasound receiver. In particularly preferred devices, the energy source comprises a laser and wherein at least one of the energy source and the energy detector is operationally coupled to the fluid receiving element via an optical fiber.

It should further be appreciated that the fluid is a reagent for analysis of a biological sample or a biological fluid, and suitable fluids may also include an additive selected from the group consisting of a dye, a fluorophor, a phosphorescent compound, and a luminescent compound (e.g., chemi-, bio-, or electroluminescent).

The processor is contemplated to processes a signal from an analysis section that analyzes binding of an analyte to a biochip, wherein the volume of the fluid is preferably determined by a user. The pipette may further comprise a data transfer interface that electronically couples the pipette to a person other than a user of the pipette.

In especially preferred aspects, the fluid receiving element removes the fluid from a container and dispenses at least part of the fluid to a biochip within an analytic device, and it is particularly contemplated that the fluid receiving element is coupled to a plurality of actuators that move the fluid receiving element along at least one of an x-axis, a y-axis, and a z-axis.

In still another aspect of the inventive subject matter, contemplated devices may comprise a detector that acquires an analyte signal from an analyte that is bound to a substrate on a biochip, wherein the detector has a focal plane that is determined using a reference signal from a reference marker on the biochip. It is generally preferred that the detector comprises a confocal microscope or a dark field microscope, wherein the analyte

signal is selected from the group consisting of fluorescence, chemiluminescence, and phosphorescence.

Contemplated analytes are typically bound to the substrate via a crosslinker, wherein binding of the analyte may be non-covalent, and wherein the crosslinker comprises biotin. Thus, suitable analytes may comprise at least one of a peptide, a nucleic acid, and an enzyme inhibitor.

In still further contemplated aspects, a suitable reference signal may be selected from the group consisting of reflected light, fluorescence, chemiluminescence, and phosphorescence, wherein the reference marker is selected from the group consisting of a reflective marker, a fluorophor, a chemiluminescent compound, and a phosphorescent compound. It is generally contemplated that the analyte signal may be generated using a first light source, wherein the reference signal is generated using a second light source (e.g., first light source comprises a laser and the second light source comprises a light emitting diode).

In still further preferred aspects, the biochip comprises a housing that is at least partially transparent for light emitted from the second light source, and wherein the reference marker is illuminated by the second light source. Therefore it should be recognized that the reference marker may be illuminated by the second light source in a dark field. Furthermore, suitable analytic systems may include a second reference marker on the biochip, wherein the focal plane is determined using the reference marker and the second reference marker.

In a preferred mode of operation, the analyte signal is normalized using at least one of a reference beam and a positive control marker on the biochip, wherein the reference beam is generated using a beam splitter that splits a beam from a light source that illuminates the analyte. Therefore, the analytic system may also comprise at least two light sources that illuminate the analyte and an optional second analyte, wherein one of the light sources emits light having a wavelength that is at least 15 nm apart from a wavelength emitted from another one of the light sources. Where a second analyte is present it is contemplated that the detector simultaneously detects the analyte signal from the analyte and a second analyte signal from the second analyte. Further particularly preferred aspects include amplification of the signal, wherein in especially contemplated aspects the signal is

amplified using enhancers that include metals, cyclodextrins, organic solvents, and/or antibodies to the label(s).

Suitable analytical systems may further include a data transfer interface electronically coupled to the detector, wherein the data transfer interface preferably communicates with a person other than the user of the analytical system.

Thus, specific embodiments and applications of analytic systems have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. Moreover, in interpreting the specification, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

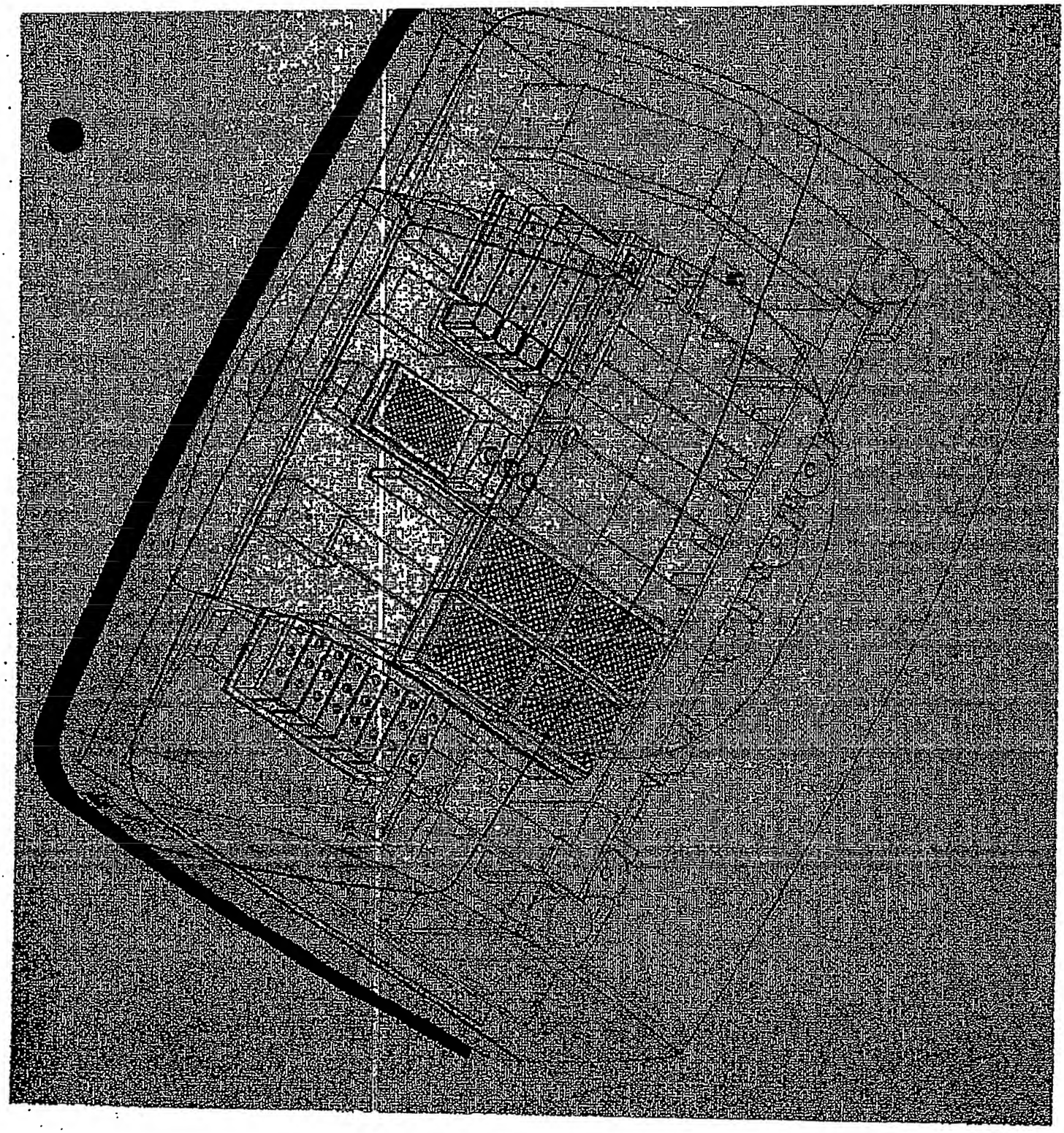

Figure 1